

REMARKS

Claims 1-5, 8, 17, 47-51, 58, 63, and 69-77 are pending in the application and have been examined. Claims 1-5, 8, 17, 47-51, 57, 63, and 69-77 stand rejected. Claims 1, 47, 58, 63, 69, 70, and 71 have been amended. No new matter has been introduced. Reconsideration and allowance of Claims 1-5, 8, 17, 47-51, 58, 63, and 69-77 are respectfully requested.

1. The Claim Objections

The Examiner has objected to Claims 58, 63, and 69 for depending from a canceled claim. The Examiner notes that Claims 58 and 63 were treated as incorporating all the limitations of canceled Claim 57, and Claim 69 was treated as incorporating all the limitations of canceled Claim 64. Claims 58 and 63 have been amended to incorporate all the limitations of canceled Claim 57, and Claim 69 has been amended to incorporate all the limitations of canceled Claim 64. Withdrawal of this ground of objection is respectfully requested.

2. The Rejection Under 35 U.S.C. § 102(b)

The Examiner has rejected Claims 1-3, 8, 17, 47-51, 63, and 69-71 under 35 U.S.C. § 102(b) as being anticipated by Kogler et al. (1996) *Biotechnol. Prog.* 12(6):822-36. According to the Examiner, no skilled or precise shaping is required by the claim language, and the general limitation "dynamic control" is met by any modification in the field in the course of the separation. Moreover, the Examiner states that the object of the limitation "dynamic control" is not to produce a dynamically shaped field. Applicants respectfully disagree.

As defined in the specification, dynamic control refers to controlling each electrode of the array individually to maintain and adjust the electric field gradient during the course of solute focusing and/or separation (Specification, page 8, lines 14-16). However, to more clearly define the claimed invention, independent Claims 1, 47, 58, 63, 69, 70, and 71, from which the Claims 2, 3, 8, 17, and 8-51 depend, have been amended to recite that the voltage applied to the

LAW OFFICES OF
CHRISTENSEN O'CONNOR JOHNSON KINDNESS^{PLLC}
1420 Fifth Avenue
Suite 2800
Seattle, Washington 98101
206.682.8100

electrode array or the electric field gradient is dynamically controlled to produce a dynamically shaped field. Support for this amendment can be found throughout the specification, for example, at page 4, lines 6-26; page 7, lines 8-15; page 21, lines 15-22; page 24, lines 13-19; page 25, lines 6-10). Applicants respectfully submit that Koegler et al. does not provide an enabling disclosure of means for dynamically controlling an electric field gradient to produce a dynamically shaped field.

For the reasons described above and the reasons provided in the Amendment and Response to the non-final Examiner's Action, filed January 3, 2005, Koegler et al. does not anticipate the claimed invention. Applicants respectfully request withdrawal of this ground of rejection.

3. The Rejection Under 35 U.S.C. § 103(a)

The Examiner has rejected Claims 4, 5, 58, 72, 74, and 76 under 35 U.S.C. § 103(a) as being obvious over Koegler et al. (1996) *Biotechnol. Prog.* 12(6):822-36 in view of U.S. Patent No. 5,298,143 (Ivory et al.). In addition, the Examiner has rejected Claims 73, 75, and 77 under 35 U.S.C. § 103(a) as being obvious over Koegler et al. in view of Ivory et al. as applied to Claim 72, and in further view of U.S. Patent No. 4,670,119 (Hurd), U.S. Patent No. 6,013,168 (Arai), or U.S. Patent No. 5,582,701 (Cabilly et al.), respectively. According to the Examiner, it would have been obvious to modify the device and method of Koegler et al. by using the linear array of electrodes and the controller comprising a plurality of controller units in communication with the electrode array disclosed in Ivory et al. Applicants respectfully disagree.

According to the Examiner, it is not apparent that the use of the array of electrodes disclosed in Ivory et al. in the device of Koegler et al. would result in severe peak smearing, as argued by the applicants in the Amendment and Response to the non-final Examiner's Action, filed January 3, 2005. Appended hereto as Attachment A is the declaration by Dr. C. F. Ivory

LAW OFFICES OF
CHRISTENSEN O'CONNOR JOHNSON KINDNESS^{PLLC}
1420 Fifth Avenue
Suite 2800
Seattle, Washington 98101
206.682.8100

("Ivory Declaration"), which explains that one of skill in the art would not have combined the array of electrodes disclosed in Ivory et al. with the device of Koegler et al., for the following reasons. One skilled in the art would have realized that the use of a linear electrode array as disclosed in Ivory et al. with the Koegler et al. device would have resulted in severe peak smearing, for the following reason: The large diameter of the dialysis tubing used in Koegler et al. (6 mm diameter, see Koegler et al., page 828, Column 2, first paragraph in "Experimental Results") would cause the proteins to be radially pulled away from the center of the dialysis tube to form a thin layer against the wall of the dialysis tube, which would then smear out perpendicularly (Ivory Declaration, paragraph 5). This smearing could be reduced but not eliminated by the use of a set of ring electrodes (Ivory Declaration, paragraph 5). Dialysis tubing with smaller diameters were not available at the time of publication of Koegler et al. Therefore, one of skill in the art would not have been motivated to combine any of the electrode arrays of Ivory et al. with the device of Koegler et al. to produce a dynamically shaped field (Ivory Declaration, paragraph 5).

In addition, there is objective evidence of non-obviousness of the claimed invention. After the time of publication of the Koegler et al. article, others in the field were skeptical that dynamic field gradient focusing would work (Ivory Declaration, paragraph 6). Moreover, others have copied and are using the claimed device, as described in the Ivory Declaration (Ivory Declaration, paragraph 7).

For the above reasons, and the reasons provided in the Amendment and Response to the non-final Examiner's Action, filed January 3, 2005, the cited references, either alone or in combination, fail to teach, suggest, provide any motivation to make, or otherwise render obvious the claimed invention. Withdrawal of this ground of rejection is respectfully requested.

LAW OFFICES OF
CHRISTENSEN O'CONNOR JOHNSON KINDNESS^{LLC}
1420 Fifth Avenue
Suite 2800
Seattle, Washington 98101
206.682.8100

4. The Obviousness-Type Double Patenting Rejection

The Examiner has rejected Claims 1-5, 8, 17, and 70 under the doctrine of obviousness-type double patenting as being unpatentable over Claims 7-12 and 21 of U.S. Patent No. 6,277,258 in view of Ivory et al. or Koegler et al. In addition, the Examiner has rejected Claims 47-51 and Claim 58 under the doctrine of obviousness-type double patenting as being unpatentable over Claims 32-36 and Claim 37, respectively, of U.S. Patent No. 6,277,258 in view of Ivory et al. or Koegler et al. The Examiner has also rejected Claims 72, 74, and 76 under the doctrine of obviousness-type double patenting as being unpatentable over Claim 7 of U.S. Patent No. 6,277,258 in view of Ivory et al. or Koegler et al. Furthermore, the Examiner has rejected Claims 63 and 69 under the doctrine of obviousness-type double patenting as being unpatentable over Claims 38 and 42 in view of Claim 25 of U.S. Patent No. 6,277,258. Moreover, the Examiner has rejected Claim 71 under the doctrine of obviousness-type double patenting as being unpatentable over Claim 32 of U.S. Patent No. 6,277,258. Also, the Examiner has rejected Claims 72, 74, and 76 under the doctrine of obviousness-type double patenting as being unpatentable over Claim 7 of U.S. Patent No. 6,277,258 in view of Ivory et al. or Koegler et al. Finally, the Examiner has rejected Claims 73, 75, and 77 under the doctrine of obviousness-type double patenting as being unpatentable over Claim 7 of U.S. Patent No. 6,277,258 in view of Ivory et al. or Koegler et al. and in further view of Hurd; Arai, and Cabilly et al., respectively. A terminal disclaimer over U.S. Patent No. 6,277,258 is enclosed with this response. Withdrawal of this ground of rejection is respectfully requested.

Conclusion

In view of the above amendments and foregoing remarks, applicants believe that Claims 1-5, 8, 17, 47-51, 58, 63, and 69-77 are in condition for allowance. If any issues remain

LAW OFFICES OF
CHRISTENSEN O'CONNOR JOHNSON KINDNESS^{PLLC}
1420 Fifth Avenue
Suite 2800
Seattle, Washington 98101
206.682.8100

that may be expeditiously addressed in a telephone interview, the Examiner is encouraged to telephone applicants' attorney at 206.695.1783.

Respectfully submitted,

CHRISTENSEN O'CONNOR
JOHNSON KINDNESS^{PLLC}



Karen Blöchlinger, Ph.D.
Registration No. 41,395
Direct Dial No. 206.695.1783

I hereby certify that this correspondence is being deposited with the U.S. Postal Service in a sealed envelope as first class mail with postage thereon fully prepaid and addressed to Mail Stop RCE, Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450, on the below date.

Date: 6/23/05

Carole Julian

KBB:cj

LAW OFFICES OF
CHRISTENSEN O'CONNOR JOHNSON KINDNESS^{PLLC}
1420 Fifth Avenue
Suite 2800
Seattle, Washington 98101
206.682.8100

Attachment A

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Applicants: C.F. Ivory et al. Attorney Docket No.: WSUR117329
Application No.: 09/885,439 Group Art Unit: 1753
Filed: June 19, 2001 Examiner: J.T. Barton
Title: DEVICE AND METHOD FOR FOCUSING SOLUTES IN AN ELECTRIC
FIELD GRADIENT

DECLARATION OF DR. C.F. IVORY

Seattle, Washington 98101

June 15, 2005

TO THE COMMISSIONER FOR PATENTS:

I, Dr. Cornelius F. Ivory, declare as follows:

1. I am a co-inventor of the subject matter in the above-identified application.
2. The following is a summary of my educational and employment background. I received a B.S. degree in Chemical Engineering from the University of Notre Dame in 1974, an M.A. degree in Chemical Engineering from Princeton University in 1976, and a Ph.D. degree in Chemical Engineering from Princeton University in 1980. I was an Assistant Professor of Chemical Engineering at the University of Notre Dame from 1980 to 1987 and I've been a Professor of Chemical Engineering at Washington State University since 1987. Since 2001, I've been the Associate Director of the NIH Predoctoral Program in Biotechnology. I have more than 25 years experience in the field of electrophoretic devices and methods, starting as a visiting scientist in NASA's bioseparations group at Marshall Space Flight Center and continuing as a full-time faculty member at several U.S. universities, including experience in analyzing, designing, fabricating, and testing electrophoresis instruments, as well as inventing several new instruments and developing new electrophoresis protocols. A copy of my *curriculum vitae* is appended hereto as Attachment B.

LAW OFFICES OF
CHRISTENSEN O'CONNOR JOHNSON KINDNESSSM
1420 Fifth Avenue
Suite 2800
Seattle, Washington 98101
206.682.8100

3. I have read the Office Action mailed on March 10, 2005, concerning the above-identified patent application and reviewed the references cited therein. I am a co-author of Koegler et al. (1996) *Biotechnol. Prog.* 12(6):822-36 and a co-inventor of U.S. Patent No. 5,298,143 (Ivory et al.). The Office Action states that it would have been obvious to one of skill in the art to modify the device and method of Koegler et al. by using the linear array of electrodes and the controller comprising a plurality of controller units in communication with the electrode array disclosed in Ivory et al.

4. I and my co-workers intentionally never tried to use the array of electrodes described in Ivory et al. with the device described in Koegler et al. because we thought that this would work poorly, if at all. Specifically, because of the large diameter of the dialysis tube used in the device of Koegler (i.e., 6.4 mm), using any of the arrays of electrodes disclosed in Ivory et al. (even a set of ring electrodes) with the Koegler et al. device would have caused the proteins to be radially pulled away from the center of the dialysis tube to form a thin layer against the wall of the dialysis tube. This thin layer would then smear out perpendicularly. The smearing would be particularly severe if a linear electrode array were used, but even a set of ring electrodes would cause smearing of the protein peaks using the large-diameter dialysis tubing used in Koegler et al. Dialysis tubes with smaller diameters were not available at the time of the publication of Koegler et al. This would have been known to others in this field and would have discouraged them from combining the electrode array in Ivory et al. with the Koegler et al. device.

5. Other people in this field were skeptical that we would be able to achieve dynamic control of the electric field gradient. This skepticism was expressed in reviews of grant proposals I submitted after the publication of Koegler et al. For example, in review of a grant proposal I submitted together with Dr. Derek McLean, the reviewer stated that "the DFGF

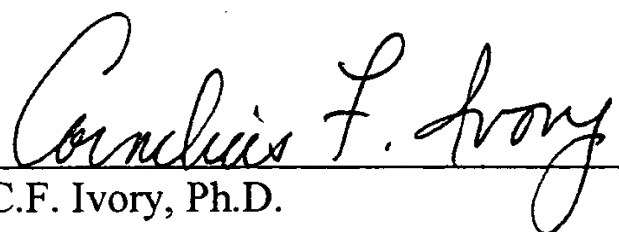
LAW OFFICES OF
CHRISTENSEN O'CONNOR JOHNSON KINDNESS^{LLC}
1420 Fifth Avenue
Suite 2800
Seattle, Washington 98101
206.682.8100

[dynamic field gradient focusing] method has not yet been shown to be a viable method for fractionating integral membrane proteins" (Summary Statement, Application No. RO3 HD045370-01, page 5, second paragraph; enclosed as Attachment C).

6. Although the claimed devices are not yet commercially available, several other groups have now produced similar devices, with my help and advice. These groups include Professor Milton Lee's group in the Chemistry Department at Brigham Young University in Utah, Professor Richard Ansell's group in the Chemistry Department at Leeds University in England, and Dr. Peter Myers' group at Leeds University and York University in England.

7. All statements made herein and of my own knowledge are true, and all statements made on information and belief are believed to be true; and further, these statements were made with the knowledge that willful, false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful, false statements may jeopardize the validity of the above-identified application or any patent issued thereon.

Respectfully submitted,


C.F. Ivory, Ph.D.

Date:

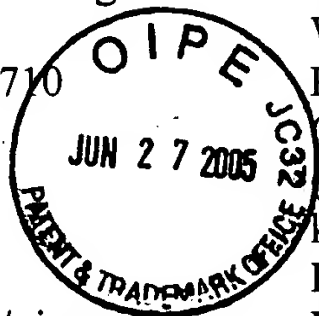
KB/cj

June 15, 2005

LAW OFFICES OF
CHRISTENSEN O'CONNOR JOHNSON KINDNESS^{PLC}
1420 Fifth Avenue
Suite 2800
Seattle, Washington 98101
206.682.8100

Attachment B

Spouse: Prof. Kwan Hee Kim
431 Heald Hall
Dept. Genetics and Cell Biology
Washington State University
Pullman, Washington 99164-4234
(509) 335-7022 Work
(509) 335-9688 FAX
khkim@wsu.edu
Birthdate: 2/19/52
URL: <http://www.wsu.edu:8080/~khkim/>



Program Co-Chair, American Electrophoresis Society, 11/03 National Meeting in San Francisco
Panelist, NSF CAREER review panel on Biotechnology, 12/01, 12/02
Editorial Board, *Separation Science and Technology*, 1/97-present.

Head, USRA-NASA review panel on Microgravity Biotechnology, 12/96, 7/98, 1/01.

Invited to write book chapter for "*The Handbook of Isoelectric Focusing and Proteomics*" F02.

Invited to write book chapter for "*The Encyclopedia of Bioprocess Technology*," F97.

Invited to write book chapter for "*Separation Processes in Biotechnology*," F96.

Electroseparations 2020 Workshop Sponsored by NSF, EPRI and DOE, October 9-11, 1995.

PNL Affiliate Staff Scientist (PASS, Battelle Pacific Northwest Labs) 1994-1997.

Outstanding Professor in Chemical Engineering (Undergraduate Academic, WSU) 1990.

Editorial Advisory Board, Industrial and Engineering Chemistry Research, 1990-1991.

Special Issue Editor, *Separation Science and Technology*, Special Issue on Bioseparations, Vol. 23(8-9), Marcel Dekker, Inc., New York 1988.

National Program Committee Chair for Industrial and Engineering Chemistry Division of the American Chemical Society, 1987-1991.

Panelist, National Science Foundation, Minority Graduate Fellowship Evaluation Panel in Engineering, Served: 1985, 1987, 1989.

NASA-USRA Visiting Scientist, Bioseparations Branch at Marshall Space Flight Center, Huntsville, Alabama, 1/79 - 2/80.

5. PROFESSIONAL ACTIVITIES.

Consulting: Intel Corp., Fluor-Daniels, Inc., Protasis Corp., MetapHoresis Corp., Calibrant Technol.

American Electrophoresis Society, 2001-present, Program Chair, 2003 Fall Meeting.

American Chemical Society (I&EC, BIOT & Analytical Chemistry Divisions).

National Program Committee Chair, I&EC Division of the ACS, 1987-90.

6. COLLABORATORS.

Prof. Abraham **Lenhoff**, Dept. Chemical Engng, U. Delaware, Newark DE, Rockville MD: Nonlinear ionic transport theory applied to ion exchange

Dr. Jürgen **Hubbuck** Head, Downstream Processing, Inst. Enzyme Technol., Heinrich-Heine University: Nonlinear ionic transport theory applied to ion exchange.

Prof. Cheng S. **Lee**, Dept. Chem. & Biochem., U. Maryland, College Park and Calibrant Technologies, Rockville MD: Recovery of low abundance proteins for proteomic analysis using isotachopheresis.

Prof. Richard **Ansell**, School of Chemistry, University of Leeds: Commercial applications of dynamic focusing to non-amphoteric, low molecular weight organic molecules.

Mr. David **Strand**, CEO, Protasis Corp., Marlborough MA: Commercialization of dynamic focusing apparatus.

Dr. David **Ross**, NIST, Gaithersburg; Prof. **Van Cott**, VPI: Simulation of electrophoresis in a diverging channel.

Dr. Brent **Larsen**, Berlex Corp., Mountaintop CA: Preparative electrofocusing.

Dr. Scott **Sibbet**, INTEL Corp., Rio Rancho NM: Microchannel electrophoresis.

Prof. P. **Dutta**, WSU: Isoelectric focusing of proteins in a polymeric chip.

Prof. B. **Van Wie**, WSU, Prof. J. **Cheng**, WSU, Prof. D. **Moffett** WSU: Bioamplified Sensors.

Prof. G. **Lopez**, H. **Sang**, S. **Brueck**, UNM (NIRT): Fundamentals of nanometer scale separation chips.

7a. INVITED LECTURES AND SYMPOSIA.

Universities and Institutes...

Brigham Young University, Provo, UT, 4/2/04, "Preparative Electrofocusing at 10 kV."

NIST (Gaithersburg), 12/10/03 "How to Scale Capillary Electrofocusing by 1,000,000x"

University of Delaware, Newark, DE, 10/23/03, "How to Scale Capillary Electrofocusing by 1,000,000x."

University of Alberta, Alberta, Canada, 9/24/01, "Advances in Electrofocusing."

University of Idaho, Moscow, Idaho, 10/16/97, "Isoelectric Focusing at High Voltages."
Virginia Polytechnic Institute, Blacksburg, Virginia, 1/28/92, "Preparative-Scale Electrophoresis."
University of Idaho, Moscow, Idaho, 9/23/87; "Toward Industrial Electrophoresis of Proteins."
University of Arizona, Tucson, Arizona (Center for Separation Science, 1/15/87; "Magnetic Stabilization of Weakly Conducting Fluids."
UCLA, Los Angeles, California, 1/16/87; "Magnetic Stabilization of Weakly Conducting Fluids."
University of Iowa, Iowa City, Iowa, 4/17/87; "Magnetic Stabilization of Weakly Conducting Fluids."
University of Colorado, Boulder, Colorado, 2/13/86; "Stable, Unstable and Stabilized Natural Convection in Continuous Flow Electrophoresis."
Northwestern University, Evanston, Illinois, 5/14/85; "Large-Scale Continuous Flow Electrophoresis."
Yale University, New Haven, Connecticut, 1/25/85; "High-Resolution Continuous Flow Electrophoresis."
Northwestern University, Evanston, Illinois, 9/17/83; "The Effect of Electric Fields on Carrier-Mediated Transport."
KAIST (Korea Advanced Institute for Science and Technology) Seoul, Korea, 5/27/83; "Scale-up of the Free-Flow Electrophoresis Device."
Korea University, Seoul, Korea, 5/26/83; "Using Electric Fields to Control Transmembrane Fluxes."
Seoul National University, Seoul, Korea, 5/25/83; "Continuous Flow Electrophoresis with Solute Recycle."

Interdepartmental Seminars...

Dept. Chemistry, Washington State University, Pullman, Washington, 4/04/03; "Electrophoresis in Proteomics."
 Biosystems Engng, Washington State University, Pullman, Washington, 3/08/00; "How Electrophoresis is Used in Bioprocessing."
 Dept. Chemistry, Washington State University, Pullman, Washington, 10/11/93; "Industrial-Scale Electrophoresis."
 NIH Biotechnology Symposium, Washington State University, Pullman, Washington, 10/13/90, "New Purification Technologies for Proteins: A Chemical Engineering Approach."
 Dept. Agricultural Engineering, Washington State University, Pullman, Washington, 1/12/89; "Electrophoresis in Downstream Processing."
 Dept. Physics, Washington State University, Pullman, Washington, 1/12/88; "Electrically-Induced Secondary Flows."
 Dept. Mechanical Engineering, University of Notre Dame, Notre Dame, Indiana, 3/23/86; "Stable, Unstable and Stabilized Natural Convection in an Electrically-Heated Slit."

Industrial Seminars...

Calibrant Biosystems, Rockville MD, 12/12/2003; "Tutorial on Modeling Isotachophoresis." C. F. Ivory
Calibrant Biosystems, Rockville MD, 11/1/2002; "Preparative Isotachophoresis." C. F. Ivory
Genentec, Point San Bruno CA 10/30/2002; "Dynamic Electrofocusing." C. F. Ivory
Applied Biosystems International, Framingham MA, 1/16/2002; "Dynamic Electrofocusing." C. F. Ivory
Immunex, Inc, Seattle, Washington, 6/22/2001; "Advances in Electrofocusing." C. F. Ivory
Ecotas '2000 in London, U.K., October 6, 2000, "Microfabricated Electrofocusing Devices," C. F. Ivory.
Ecotas '99 in Sherborn, MA, October 8-9, 1999, "Electronically-Controlled Electrofocusing," C. F. Ivory.
Waters Corporation, New Milford, MA, Sept. 9, 1998, "Electrophoretic Focusing without Ampholytes," Z. Huang and C. F. Ivory.
Immunex, Inc, Seattle, Washington, 8/19/97; "Isoelectric Focusing at High Voltages."
Battelle Pacific Northwest Laboratory, Richland, Washington, 6/4/96 Purification and Concentration of Charged Molecules using Electronically-Controlled Electric Field Gradients."

Protein Design Labs, Inc., Mountain View, CA, 5/26/94; "New Electrofocusing Technologies for Analytical, Preparative and Industrial Protein Processing."

Applied Biosystems, Inc., Seattle, CA, 5/25/94; "Electrophoretic Focusing Without pH Gradients."

Immunex, Inc., Seattle, Washington, 4/29/94; "New Electrofocusing Technologies for Analytical, Preparative and Industrial Protein Processing."

ZymoGenetics, Seattle, Washington, 4/28/94; "Electrophoretic Focusing Without pH Gradients."

ZymoGenetics, Seattle, Washington, 6/11/93; "Industrial Electrophoresis."

Battelle Pacific Northwest Laboratory, Richland, Washington, 6/3/93; "Electro-Ultrafiltration."

Genentec, Inc., South San Francisco, California, 11/25/91; "A New Apparatus for Free-Fluid Electrophoresis."

Battelle Pacific Northwest Laboratory, Richland, Washington, 5/14/90; "Zone Electrophoresis in Downstream Processing."

PPG, Pittsburgh, Pennsylvania, 6/23/89; "Preparative High Performance Capillary Electrophoresis."

PPG, Pittsburgh, Pennsylvania, 7/28/88; "Electrophoresis and Electrochromatography."

E. I. DuPont de Nemours, Wilmington, Delaware, 7/30/85; "Industrial-Scale Continuous Flow Electrophoresis."

Dow Chemical Company, Midlands, Michigan, 4/4/85; "Industrial-Scale, High Resolution, Continuous Flow Electrophoresis."

Georgia Kaolin, Dry Branch, Georgia, 3/21/85; "Scale-Up of Electrostatic Beneficiation and Dewatering Processes for Kaolin."

Battelle Memorial Laboratory, Columbus, Ohio, 12/18/84; "High Resolution, Continuous Flow Electrophoresis."

Cetus Corporation, Emeryville, California, 11/23/84; "Continuous Flow Electrophoresis with Recycle."

Cutter Laboratories, Berkeley, California, 11/21/84; "Mathematical Modeling of Diffusional Effects in the Philpot-Harwell Device."

Miles Laboratories, Elkhart, Indiana, 1/17/84; "The Application of Electrophoresis in Industrial Separations."

Invited Papers

28th International Symposium on Capillary Chromatography and Electrophoresis in Las Vegas, May 18-22, 2005, "Electrofocusing Proteins in a Velocity Gradient," C. F. Ivory.

18th International Symposium on Microscale Bioseparations in New Orleans, February 12-17, 2005, "Performance Bottlenecks in Field-Gradient Focusing: Symptoms and Solutions," C. F. Ivory and J. M. Burke.

Plenary: 27th International Symposium on Capillary Chromatography and Electrophoresis in Riva del Garda, Italy, May 31-June 4, 2004, "Dynamic Electrofocusing at Milligram Scales," C. F. Ivory.

American Electrophoresis Society, San Francisco CA, Nov. 16-20, 2003, Session on "Emerging Technologies: Electrokinetic Separations, "Separation, Concentration and Manipulation of Proteins using a Computer-Controlled Electrode Array," C. F. Ivory. (*Invited Speaker*)

Plenary: 26th International Symposium on Capillary Chromatography and Electrophoresis in Las Vegas, May 18-22, 2003, "Electrofocusing Non-Amphoteric Ionic Solutes," C. F. Ivory.

American Electrophoresis Society, Indianapolis IN, Nov. 3-6, 2002, Session on "Applications of Electrophoresis, "Extreme Electrofocusing," C. F. Ivory. (*Invited Speaker*)

23rd International Symposium on Chromatography, Olympia, London, U.K., Oct. 1-5, 2000, Session on "Capillary Electrophoresis and Electrochromatography, "Dynamic Electrofocusing," C. F. Ivory. (*Invited Speaker*)

Gordon Conference, Colby-Sawyer College, So. New London, N.H., July 31– August 5, 1994, Separation and Purification, "Electrophoresis in Downstream Processing," C. F. Ivory. (*Invited Speaker*)

Keystone Symposium on Molecular and Cellular Biology, in Santa Fe, New Mexico, Jan. 15–21, 1993, Symposium on Protein Purification and Biochemical Engineering, “Electrophoresis in Bench–top and Large Scale Processing,” C. F. Ivory, *Plenary Speaker*.

US/Germany Workshop on Downstream Processing of High-Value Proteins, Goslar, Germany, October 24-25, 1991. Preparative-Scale Electrophoresis. *Organizers*: D. I. C. Wang, H. Hustedt and K. H. Kroner.

PACHEC '88 Meeting in Acapulco, Mexico, October 19-22, 1988, Symposium on Liquid Chromatography Applied in Biological Separations, “Electrochromatography,” C. F. Ivory.

National and International Symposia...

ACS National Meeting in San Diego, March 13-18, 2005, Symposium on Systems Biotechnology: Developments and Applications, “Multi-stage isoelectric focusing in a polymeric microfluidic chip,” **Huanchun Cui** and C. F. Ivory.

ACS National Meeting in San Diego, March 13-18, 2005, Symposium on Systems Biotechnology: Developments and Applications, “Multi-stage isoelectric focusing in a polymeric microfluidic chip,” Huanchun Cui and C. F. Ivory.

ACS National Meeting in Anaheim, March 28-April 1, 2004, Symposium on High-Resolution Purification and Chromatography, “Role of Interior Electrical Potentials in Ion Exchange,” C. F. Ivory.

ACS National Meeting in Anaheim, March 28-April 1, 2004, Symposium on High-Resolution Purification and Chromatography, “True moving-bed electrophoresis: Increasing the scale of binary enantiomer separations by using RO to reduce solvent volumetric flows,” Brian Thome and C. F. Ivory.

ASME IMECE Annual Meeting, in Washington DC, Nov 15-21, 2003 “High Resolution Separation of Proteins in a Polymeric Micro-Fluidic Chip.” P. Dutta (*speaker*), K. Horiuchi, H. Cui and C. F. Ivory

AIChE Annual Meeting, in San Francisco, Nov 16-22, 2003 “Modeling Dynamic Field Gradient Focusing.” Noah Tracy and C. F. Ivory

AIChE Annual Meeting, in San Francisco, Nov 16-22, 2003 “Debottlenecking of Electrofocusing for use in Proteomics.” Jeff Burke and C. F. Ivory

ACS National Meeting in Boston, August 18-23, 2002, Symposium on Advances in Bioseparations, “Enantiomer Separations by Continuous Electrophoresis,” Brian Thome and C. F. Ivory.

AIChE Annual Meeting in Reno, Nov. 4-8, 2001, Symposium on Advances in Electrophoresis I: Fundamentals, “Protein Fractionation using Automated Electric Field Gradient Focusing,” Zheng Huang and C. F. Ivory.

AIChE Annual Meeting in Reno, Nov. 4-8, 2001, Symposium on Advances in Electrophoresis II: Materials and Methods, “Advances in Electrofocusing,” C. F. Ivory and Noah Tracy.

AIChE Annual Meeting in Reno, Nov. 4-8, 2001, Symposium on Advances in Biotech. Processing, “Enantiomer Pair Processing by Continuous Electrophoresis,” Brian Thome and C. F. Ivory.

ACS National Meeting in San Diego, April 1-5, 2001, Symposium on Biological Separations -Theory and Practice Symposium, “Enantiomer Separations by Continuous Electrophoresis,” C. F. Ivory.

ACS National Meeting in San Francisco, March 26-30, 2000, Symposium on Bioseparations, “Preparative Isoelectric Focusing at High Voltages,” C. F. Ivory.

ACS National Meeting in Anaheim, March 21-25, 1999, Symposium on Bioseparations, “Electrophoretic Focusing without Ampholytes,” Z. Huang and C. F. Ivory.

ACS National Meeting in San Diego, March 13-18, 1994, Symposium on Preparative Bioseparations, “Electrophoretic Focusing without Ampholytes,” C. F. Ivory, W. S. Koegler, and W. A. Gobie.

ACS National Meeting in San Diego, March 13-18, 1994, Symposium on Electrophoretic Bioseparations, “Electric Field–Gradient Focusing,” C. F. Ivory, W. S. Koegler, R. D. Greenlee and V. Surdigio.

AIChE Annual Meeting in Miami, Nov. 1-6, 1992, Transport Phenomena in Bioseparations-I, “High Performance Electrophoresis,” C. F. Ivory, W. Koegler and W. A. Gobie.

- Third International Symposium on Field-Flow Fractionation in Park City, Utah, October 3-4, 1992, "A Hybrid Rotor for Continuous Bioprocessing" C. F. Ivory, M. Gilmartin, W. A. Gobie, C. A. McDonald and R. L. Zollars.
- ACS National Meeting in San Francisco, April 5-9, 1992, Symposium on Preparative Bioseparations, "Electro-Ultrafiltration," C. F. Ivory, and W. A. Gobie.
- AIChE Annual Meeting in Los Angeles, Nov. 17-22, 1991, Symposium on Downstream Processing-II, "A New Apparatus for Free-Fluid Electrophoresis," W. A. Gobie and C. F. Ivory.
- AIChE Annual Meeting in Los Angeles, Nov. 17-22, 1991, Symposium on Transport Phenomena in Bioseparations-II, "Electrically Driven Ultrafiltration," W. A. Gobie and C. F. Ivory.
- ACS National Meeting in Boston, April 22-27, 1990, Symposium on Advances in Chromatography, "Continuous Centrifugal Field-Flow Fractionation," C. F. Ivory, R. L. Zollars and C. A. McDonald.
- ACS National Meeting in Boston, April 22-27, 1990, Symposium on Chromatography and Separation Science Emphasizing Biological Separations. IV Preparative and Large-Scale Electrophoresis, "Free-Flow Zone Electrophoresis in Downstream Processing," C. F. Ivory and W. A. Gobie.
- ACS National Meeting in Boston, April 22-27, 1990, Symposium on Chromatography and Separation Science Emphasizing Biological Separations. V. Electrochromatography, "Counteracting Chromatographic Electrophoresis (CACE)," W. A. Gobie and C. F. Ivory.
- ASME Winter Annual Meeting in San Francisco, Dec. 11 - 15, 1989, Symposium on Fluid Dynamics: Bioprocess Engineering, "Zone Electrophoresis in Downstream Processing," C. F. Ivory and W. A. Gobie.
- AIChE Annual Meeting in San Francisco, Nov. 5-10, 1989, Symposium on Separation of Bioactive Compounds, "Mathematical Models for Gel Permeation Chromatography and Gel Electrochromatography," C. F. Ivory and T. Adhi.
- AIChE Annual Meeting in San Francisco, Nov. 5-10, 1989, Symposium on Concentration from Dilute Solution, "Countercurrent Electrochromatography," C. F. Ivory and W. A. Gobie.
- ACS National Meeting in Miami, September 11-15, 1989, Symposium on New Advances in Protein Purification, "Analytical, Preparative and Large-Scale Zone Electrophoresis," C. F. Ivory and W. A. Gobie.
- AIChE Annual Meeting in Washington, D.C., Nov. 27 - Dec. 2, 1988, Symposium on Transport Processes in Bioseparation Systems, "Electrochromatography," C. F. Ivory and W. A. Gobie.
- WEST '88 Meeting in Seattle, Washington, October 17-18, 1988, Symposium on Biotechnology, "Continuous Purification with Electrical and Centrifugal Fields," C. F. Ivory.
- ACS National Meeting in Toronto, June 5-11, 1988, Symposium on Large-Scale Separations, "A State-of-the-Art Review of Electrophoresis in Downstream Processing," C. F. Ivory.
- ACS National Meeting in Toronto, June 5-11, 1988, Symposium on Novel Bioseparations, "Kinetic and Electrostatic Enhancement of Facilitated Transport of Biomolecules," L. A. Dall-Bauman and C. F. Ivory.
- AIChE Annual Meeting in New York, November 15-20, 1987, Symposium on Recent Advances in Separation Science-I, "Affinity-Mediated Protein Transport," L. A. Dall-Bauman and C. F. Ivory.
- NAMS First Annual North American Meeting in Cincinnati, June 3-5, 1987, "Kinetic and Electrostatic Enhancement of Facilitated Transport of Biomolecules" L. A. Dall-Bauman and C. F. Ivory.
- AIChE Annual Meeting in New York, November 15-20, 1987, Symposium on Recent Advances in Separation Techniques, "Continuous Counteracting Chromatographic Electrophoresis," C. F. Ivory and W. A. Gobie.
- AIChE Annual Meeting in New York, November 15-20, 1987, Symposium on Protein and Peptide Purification: New Engineering Approaches, "Recycle Continuous Flow Electrophoresis: Theory and Experiment," W. A. Gobie and C. F. Ivory.
- AIChE Annual Meeting in New York, November 15-20, 1987, Symposium on Fundamental Research in Heat and Mass Transfer, "Pattern Formation in Conductive Films," R. S. Turk and C. F. Ivory.

- AICHE Annual Meeting in Miami, November 2-10, 1986, Symposium on Recent Advances in Separation Science-I, "Magnetic Stabilization of the Continuous Flow Electrophoresis Device," C. F. Ivory, J. B. Beckwith, W. A. Gobie, R. Hergenrother and M. Malec.
- ACS National Meeting in Anaheim, Sept. 7-12, 1986, Symposium on Advances in Biological Separations Technology, "A Mathematical Model of the Annular Continuous Flow Electrophoresis Device," C. F. Ivory and J. B. Beckwith.
- AICHE Annual Meeting in Chicago, November 10-14, 1985, Symposium on Membrane Separations, "Coupling of Charge and Mass Fluxes in Facilitated Transport Membranes for Gas Separations," C. F. Ivory, P. M. Gallagher and A. L. Athayde.
- ACS National Meeting in Chicago, Sept. 8-13, 1985, Symposium on Recovery of Fermentation Products, Recent Advances and Mathematical Models, "High Resolution, High Yield Continuous Flow Electrophoresis," W. A. Gobie and C. F. Ivory.
- AICHE Annual Meeting in Chicago, November 10-14, 1985, Symposium on Fundamental Research in Heat and Mass Transfer, "Diffusion-Reaction Problems with Partial Reaction Equilibrium: Solution by Composite Flux Technique," P. M. Gallagher, A. L. Athayde and C. F. Ivory.
- ACS National Meeting in Chicago, Sept. 8-13, 1985, Symposium on Recent advances in Membrane Separation Science, "The Effect of AC Electric Fields on Carrier-Mediated Transport," A. L. Athayde and C. F. Ivory.
- AICHE National Meeting in San Francisco, November 25-30, 1984, Symposium on Recent Advances in Biotechnology, "High Resolution Free-Flow Electrophoresis," W. A. Gobie, J. B. Beckwith and C. F. Ivory.
- AICHE Annual Meeting in Anaheim, California, May 20-23, 1984, Symposium on Recent Developments in Separation Technology, "The Effect of Electric Fields on Facilitated Transport," A. L. Athayde and C. F. Ivory.
- Electrophoresis '83 Meeting in Tokyo, Japan, May 9-12, 1983, Sponsored by the Electrophoresis Society, Inc., "Scale-Up of the Free Flow Electrophoresis Device," C. F. Ivory.
- AICHE National Meeting in Los Angeles, November 14-18, 1982, Symposium on Recent Advances in Membrane Research, "Electrically Forced Facilitation in Carrier-Mediated Membrane Transport," C. F. Ivory.
- AICHE National Meeting in San Francisco, November 25-29, 1979, Symposium on Fundamental Research in Heat and Mass Transfer, "Measurement of Rate Coefficients in Slurries of Porous Spheres," C. F. Ivory.

Posters...

- ACS National Meeting in Anaheim, March 28-April 1, 2004, Analytical Chemistry "Isoelectric focusing in a polymeric micro-fluidic chip," Huanchun Cui and C. F. Ivory.
- AICHE Annual Meeting, in San Francisco, Nov 16-22, 2003 "Modeling Dynamic Field Gradient Focusing," Noah Tracy and C. F. Ivory
- AICHE Annual Meeting, in San Francisco, Nov 16-22, 2003 "Debottlenecking of Electrofocusing for use in Proteomics," Jeff Burke and C. F. Ivory
- AICHE Annual Meeting in Reno, Nov. 4-8, 2001, Poster Session: Electrophoresis Society and Awards Session, "Isoelectric Focusing using a Defined Buffer," Noah Tracy and C. F. Ivory.
- Recovery of Biological Products X in Cancún, Mexico, June 3-8, 2001, "Separation of Stereoisomers by Continuous Flow Electrophoresis," C. F. Ivory and B. Thome.
- ACS National Meeting in San Diego, April 1-5, 2001, Poster Session, "Electronically-Controlled Electrofocusing," Z. Huang and C. F. Ivory.
- HPLC2000 in Seattle, June 23-30, 2000, Poster Session, "Protein Purification using a Digitally-Controlled Electric Field Gradient," Z. Huang and C. F. Ivory.

ACS National Meeting in San Francisco, March 26-30, 2000, Poster Session, "Digitally Controlled Electrofocusing," Z. Huang and C. F. Ivory.

ACS National Meeting in San Francisco, March 26-30, 2000, Poster Session, "Preparative Separation of Enantiomers by Free-Flow Electrophoresis," B. Thome and C. F. Ivory.

Recovery of Biological Products IX in Whistler, British Columbia, Canada, May 21-25, 1999, "Dynamic Electrofocusing of Proteins," Z. Huang and C. F. Ivory.

The Whitaker Foundation in Snowbird, Utah, August 16-18, 1991, Biomedical Engineering Research Conference, "Continuous Centrifugal Field-Flow Fractionation (Poster)."

HPCE '90 in San Francisco, January 29, 1990, Second International Symposium on High Performance Capillary Electrophoresis, "Temperatures in Capillary Electrophoresis (Poster)," W. A. Gobie and C. F. Ivory.

ACS National Meeting in New York, April 13-18, 1986, First International Conference on Separations Science and Technology, "Kinetic Enhancement of Carrier-Mediated Transport: The Effect of Immobilized pH Gradients on Protein Flux (Poster)," L. Dall and C. F. Ivory.

Second Annual Congress on Automation, Scale-Up and the Economics of Biological Process Engineering, February 7-8, 1985, "Large Scale Free Flow Electrophoresis (Poster)," C. F. Ivory.

7b. CHAIRED SESSIONS AND SYMPOSIA.

ACS National Meeting in San Diego CA, March 13-18, 2005, Symposium on "Combinatorial and High-Throughput Analysis of Biological Systems," Vadim Klyushnichenko (Epic Therapeutics), Jeff Varner (Genencor) and C. F. Ivory.

ACS National Meeting in Anaheim CA, March 28-April 1, 2004, Symposium on "High-Throughput Screening/Genomics and Proteomics," Huimin Zhao (U. Illinois), Jeff Varner (Genencor) and C. F. Ivory.

ACS National Meeting in Boston, August 18-23, 2002, Symposium on "Generation and Analysis of Product Variants," Fred Jacobsen (Genentec), Dana Anderson (Genentec) and C. F. Ivory.

Recovery of Biological Products VIII. Tucson, Arizona, October 20-25, 1996, Session on *Advances in Electrokinetic Separations*, Co-sponsored by the American Chemical Society, Division of Biochemical Technology and The Engineering Foundation. *Organizers*: C. F. Ivory, WSU and Pier-Giorgio Righetti, University di Milano.

ACS Division of Analytical Chemistry. San Diego, March 13-19, 1994, Symposium on *Critical Issues in Downstream Processing*, Co-sponsored by the Analytical Chemistry Division and the Biotechnology Secretariat. *Organizers*: C. F. Ivory, WSU and Bruce Compton, AutoImmune, Inc., Lexington, MA.

ACS Division of Analytical Chemistry. San Francisco, April 5-10, 1992, Symposium on Preparative Bioseparations, Co-sponsored by the Analytical Chemistry and the Agricultural Chemistry Divisions. *Organizers*: C. F. Ivory, WSU and Jean Rivier, Salk Institute.

AIChE Annual Meeting, Los Angeles, Nov. 17-22, Area 15c, "Electrokinetic Methods in Downstream Processing," *Organizers*: C. F. Ivory, WSU and T. Scott, Oak Ridge Nat'l Lab.

ACS Division of Industrial and Engineering Chemistry. Boston, April 22-27, 1990, Symposium on Analytical, Preparative and Large-Scale Electrophoresis, Co-sponsored by the Industrial and Engineering Chemistry Division and by the Analytical Chemistry Division. *Organizers*: C. F. Ivory, WSU and A. G. Ewing, Pennsylvania State University.

PACHEC '88 Meeting in Acapulco, Mexico, October 19-22, 1988, Symposium on Liquid Chromatography in Biological Separations. Sponsored by the Instituto Mexicano de Ingenieros Quimicos. *Organizer*: C. F. Ivory.

ACS Division of Industrial and Engineering Chemistry. Anaheim, Sept. 7-12, 1985: *Advances in Biological Separations Technology*, Sponsored by the Industrial and Engineering Chemistry Division and Co-Sponsored by the Microbial and Biotechnology Division. *Organizer*: C. F. Ivory with James E. Rollings, Worcester Polytechnic Inst. and T. Alan Hatton, MIT.

ACS Division of Industrial and Engineering Chemistry. Chicago, Sept. 8-13, 1985: Recent Advances in Membrane Separation Science, Sponsored by the Industrial and Engineering Chemistry Division and Co-Sponsored by the Microbial and Biotechnology Division. *Organizer:* C. F. Ivory with R. Larter, Dept. Chemistry, Purdue University, Indianapolis Campus.

7c. SHORT COURSES, WORKSHOPS AND TUTORIALS.

Lecturer on *Electrophoresis: From microscale to macroscale*, "Advances in Electrophoretic Techniques in Environmental, Material, and Biotechnology: Fundamentals and Selected Applications," November 2001, Reno, Nevada. Sponsored by the *AIChE and the Electrophoresis Society*. *Organizer:* Pedro Arce, Florida State University. *Staff:* Pedro Arce (FSU), Neil Ivory (WSU) and Nancy Stellwagon (Biochemistry, U. Iowa).

Electro-Separations 2020, Arlington, VA, October 9-11, 1995. Sponsored by EPRI and NSF. *Organizer:* C. Byers (ORNL)

Bioprocess Workshop, Seattle, Washington, October 19, 1989. Biotechnology Needs and Assets in Washington State. *Organizers:* C. F. Ivory and A. Maret.

Lecturer on *Downstream Processing of Biological Material* in workshop entitled "Interfacing Fermentation with Recombinant DNA Technology," Sheraton Hotel, August 9-10, 1986, San Francisco, Calif. Sponsored by the *Society of Industrial Microbiology*. *Organizer:* Rich Bailey, Engenics Corporation, Menlo Park, Calif. *Staff:* Harvey Blanch (Berkeley), Neil Ivory (Notre Dame), Doug Munnecke (Genencor), Terry Papoutsakis (Rice), Carol Talkington (Engenics).

Tutorial entitled "Biological Separations," Chicago Marriott, Sept. 8, 1985, Chicago, Illinois. Sponsored by the Industrial and Engineering Chemistry Division of the American Chemical Society. *Organizer:* C. F. Ivory, Dept. Chemical Engineering, University of Notre Dame.

8. GRANTS AND SPONSORED PROGRAMS.

NSF-NIRT, CTS-0404124, "Fundamental Understanding of Microfluidics for Advanced Bioseparation and Analysis" 08/04-07/09. \$280,000

Berlex Corporation, "Preparative Fractionation of Protein Isoforms," 11/03-6/04. \$104,000

NSF, CTS-0300802, "Integrated, Multistage Isoelectric Focusing on a PDMS Microchip" 6/03-6/06. \$280,000

NSF-REU, CTS-0300802, "Integrated, Multistage Isoelectric Focusing on a PDMS Microchip" 6/03-6/06. \$6,000

NSF, BIO-0096745, "Micropreparative Purification of Proteins by Dynamic Focusing," 03/01-02/04. \$344,733

NSF-REU, BIO-0096745, "Micropreparative Purification of Proteins by Dynamic Focusing," 03/01-02/04. \$12,000

NSF, BES-9970972, "Preparative Isolation and Recovery of Protein Isoforms via Electronic Focusing," 10/99-9/02. \$484,815

NSF-REU, BES-9970972, "Preparative Isolation and Recovery of Protein Isoforms via Electronic Focusing," 10/99-9/02. \$10,000; \$12,000

NSF, BES-9417239, "Focusing of Proteins in a Multi-Chamber Electro-Ultrafiltration (MEUF) Column," 7/95-6/97. \$149,760

NSF-REU, BES-9417239, "Focusing of Proteins in a Multi-Chamber Electro-Ultrafiltration (MEUF) Column," 7/95-6/97. \$10,000

NSF, CTS-9406702, "A Hybrid Rotor for Continuous Field-Flow Fractionation," 5/95-4/97. \$85,173

NSF-REU, CTS-9406702, "A Hybrid Rotor for Continuous Field-Flow Fractionation," 5/95-4/97. \$10,000

Battelle PNL, 206028-A-L2, "Membrane Separation and Recovery of Colorants," 4/94-4/95. \$20,690

Zymogenetics, "Construction of a Preparative Membrane Electrophoresis Device," 5/94-4/95. \$5,000

NSF, DIR-9014793, "Preparative High-Performance Capillary Electrophoresis," 5/90-4/91. \$34,629

The Whitaker Foundation, "Continuous Centrifugal Field-Flow Fractionation," 3/89-2/92. \$178,000
NSF, CBT-8813864, "Large-Scale Zone Electrophoresis with Solute Recycle," 11/88-10/90. \$120,161
WTC (Wash. Technol. Ctr.), "Free-Fluid Electrophoresis Applied in Bioprocessing," 7/89-12/89. \$20,000
PPG, "Preparative High Performance Capillary Zone Electrophoresis Using Colligated Hollow Fibers," 10/88-3/89. \$26,940
WTC (Wash. Technol. Ctr.), "Continuous, Free-Fluid Electrophoresis Applied in Bioprocessing," 7/88-7/89. \$20,000
NSF, CPE-8414218, "Construction of a Modified Continuous Flow Electrophoresis Device with Solute Recycle," 6/84-5/87. \$138,869
CE (Combustion Engineering, Inc.), "The Beneficiation and Dewatering of Kaolin in an Electrostatic Field," 3/85-2/86. \$10,000
NSF, CPE-8211483, "Stability Analysis of a Proposed Continuous Flow Electrophoresis Device," 10/82-3/85. \$74,804
NSF, CPE-8105154, (Research Initiation) "Internal Staging of the Continuous Flow Electrophoresis Device with Solute Recycle," 3/81-9/83. \$48,000
EPA, IWERC Project 8003/4, "Process Modifications/New and Innovative Industrial Processes for Minimization of Environmental Pollutants," 11/80-1/82. \$31,419

Training Grants¹

NIGMS (NIH), "Predoctoral Research Training Grant in Biotechnology," 9/04-8/09. \$923,426
NIGMS (NIH), "Predoctoral Research Training Grant in Biotechnology," 9/99-8/04. \$923,426
NIGMS (NIH), "Predoctoral Research Training Grant in Biotechnology," 9/94-8/99. \$824,707
NIGMS (NIH), "Predoctoral Research Training Grant in Biotechnology," 9/89-8/94. \$476,846

9. REFERENCES. Upon request.

10. PUBLICATIONS IN REFEREED JOURNALS.

Cui, H., K. Horiuchi, P. Dutta and C. F. Ivory, "Multistage isoelectric focusing in a Poly(dimethylsiloxane) microfluidic chip," *Submitted* 2005.
 Petsev, D. N., G. P. Lopez, C. F. Ivory, S. S. Sibbett, "Microchip Protein Separation by Electric Field Gradient Focusing," *Submitted* 2005.
 Cui, H., K. Horiuchi, P. Dutta and C. F. Ivory, "Isoelectric focusing in a Poly(dimethylsiloxane) microfluidic chip," *Analytical Chemistry*, (2005).
 Ivory, C. F., "Preparative Free-Flow Electrofocusing in a Vortex-Stabilized Annulus," *Electrophoresis*, **25** 360-374 (2004).
 Ross, D., C. F. Ivory, L. E. Locascio, and K. E. Van Cott, "Peak Compression and Resolution for Electrophoretic Separations in Diverging Microchannels," *Electrophoresis*, **25**(21-22) 3694-3704 (2004).
 Tracy, N. and C. F. Ivory, "Modeling Two-Component Isoelectric Focusing Buffers in a Vortex-Stabilized Electrophoresis Apparatus," *Biotechnol. Progress* **20**(1) 193-199 (2004)
 Tracy, N. and C. F. Ivory, "Preparative Focusing of Proteins Using Binary Buffers in a Vortex-Stabilized, Free-Flow Apparatus," *Electrophoresis* **25**(12) 1748-1757 (2004).
 Ista, L. K., G. P. Lopez, C. F. Ivory, M. J. Ortiz, T. A. Schifani, C. D. Schwappach, and S. S. Sibbett, "Microchip Countercurrent Electro separation," *Lab-on-a-Chip*, **3**(4) 266-272 (2003)
 Thome, B. and C. F. Ivory, "Development of a Segmented Model for a Continuous Electrophoretic Moving Bed Enantiomer Separation," *Biotechnol. Progr.*, **19**(6) 1703-12 (2003)

¹ There are 21 training faculty from various life-science departments, 3 from chemical engineering. Faculty duties include instruction in core courses, supervision of lab rotations and graduate research, interaction with industrial sponsors (graduate student cooperative appointments) and participation in the seminar series.

- Thome, B. and C. F. Ivory, "Continuous Fractionation Of Enantiomer Pairs In Free Solution Using An Electrophoretic Analog Of Simulated Moving Bed Chromatography, " *J. Chromatography A* **953** 263-277 (2002).
- C. F. Ivory, "Temperature Profiles in the Thermal Entrance Region of Electrically-Heated, Laminar Flow in a Slit, *Chemical Engineering Science*, **55** 601-613 (2000).
- C. F. Ivory, A Brief Review of Alternative Electrofocusing Techniques, *Separation Science and Technology*, **35**(11) 1777-1793 (2000)
- Huang, Z. and C. F. Ivory, "Dynamic Field-Gradient Focusing," *Analytical Chem.*, **71**(8) 1628 (1999).
- Greenlee, R. L. and C. F. Ivory, "Focusing Proteins in a Conductivity Gradient," *Biotechnology Progress*, **14**(2) 300-309 (1998).
- Koegler, W. S. and C. F. Ivory, "Field-Gradient Focusing: A Novel Method for Protein Separation," *Biotechnology Progress*, **12**(6) 822-836 (1996).
- Koegler, W. S. and C. F. Ivory, "Focusing Proteins in an Electric Field Gradient," *J. Chromatogr. A*, **726** 229-236 (1996).
- C. F. Ivory, M. Gilmartin, C. McDonald, Gobie, W. A. and R. L. Zollars, "A Hybrid Rotor for Continuous Bioprocessing," *Biotechnology Progress*, **11**(1) 21-32 (1995).
- C. F. Ivory, "The Development of Recycle Zone Electrophoresis," *Electrophoresis*, **11** 919 (1990).
- Gobie, W. A. and C. F. Ivory, "A Thermal Model of Capillary Electrophoresis and a Method for Counteracting Thermal Band Broadening," *J. Chromatogr.*, **516**(2) 192 (1990).
- C. F. Ivory, W. A. Gobie and T. P. Adhi, "Analytical, Preparative and Large-Scale Zone Electrophoresis," in *Protein Purification*, Ladisch, M. R., R. C. Willson, C-d. C. Painton and S. E. Builder, Eds., ACS Symposium Series 427, American Chemical Society, Washington, D. C. 1990.
- Dall-Bauman, L. and C. F. Ivory, "Protein Separation via Affinity-Mediated Transport," in *Downstream Processing and Bioseparation*, Hamel, J-F. P., J. B. Hunter and S. K. Sikdar, Eds., ACS Symposium Series 419, American Chemical Society, Washington, D. C. 1990.
- Gobie, W. A. and C. F. Ivory, "Continuous Counter-Acting Chromatographic Electrophoresis," *Biotechnology Progress*, **6**(1) 21 (1990).
- C. F. Ivory, "The Prospects for Large-Scale Electrophoresis," *Separation Science and Technology: Special Issue on Bioseparations*, C. F. Ivory, ed., **23**(8-9) 875, Marcel Dekker, Inc., New York (1988).
- Gobie, W. A. and C. F. Ivory, "Recycle Continuous Flow Electrophoresis: Zero Diffusion Theory," *AIChE J.*, **34**(4) 474 (1988).
- Beckwith, J. B. and C. F. Ivory, "The Influence of Diffusion on Elution Profiles in the Philpot-Harwell Electrophoretic Separator," *Chem. Engng. Commun.*, **54**(1-6) 301 (1987).
- Ivory, C. F., W. A. Gobie, J. B. Beckwith, R. Hergenrother and M. Malec, "Electromagnetic Stabilization of Weakly Conducting Fluids," *Science*, **238**(4823) 58 (1987).
- Gallagher, P. M. and C. F. Ivory, "Electrochemical Coupling in Carrier-Mediated Membrane Transport," *J. Membrane Science*, **29** 49 (1986).
- Gobie, W. A. and C. F. Ivory, "High Resolution, High Yield Continuous Flow Electrophoresis," *Separation, Recovery and Purification in Biotechnology*, J. Asenjo and J. Hong, eds., ACS Symposium Series No. 314, Amer. Chem. Soc., New York 1986.
- Gallagher, P. M., A. L. Athayde and C. F. Ivory, "The Combined Flux Technique for Diffusion Reaction Problems in Partial Equilibrium: Application to the Facilitated Transport of Carbon Dioxide in Aqueous Bicarbonate Solutions," *Chem. Engng. Science*, **41**(3) 567 (1986).
- Athayde, A. L. and C. F. Ivory, "Electrical Pumping in Carrier-Mediated Membrane Transport," *J. Membrane Science*, **24**(3) 309 (1985).
- Athayde, A. L. and C. F. Ivory, "The Effect of AC Electric Fields on Carrier-Mediated Membrane Transport," *J. Membrane Science*, **23**(2) 241 (1985).
- Gobie, W. A., J. B. Beckwith and C. F. Ivory, "High Resolution Continuous Flow Electrophoresis," *Biotechnology Progress*, **1**(1) 60 (1985).

- Ivory, C. F., "Transient Electrophoresis of a Dielectric Sphere," *J. Colloid Interface Science*, **100**(1) 239 (1984).
- Turk, R. and C. F. Ivory, "Temperature Profiles in Plane Poiseuille Flow with Electrical Heat Generation," *Chem. Engng. Science*, **39**(5) 851 (1984).
- Ivory, C. F., "Transient Electroosmosis: The Momentum Transfer Coefficient," *J. Colloid Interface Science*, **96**(1) 119 (1983).
- Ivory, C. F., "Continuous Flow Electrophoresis. The Crescent Phenomenon Revisited-II. Nonisothermal Effects," *Electrophoresis*, **2** 31 (1981).
- Ivory, C. F., "Derivation of the Particle Size Weighting Factor in the Measurement of Rate Coefficients in Slurry Reactors," *Chem. Engng. Science*, **36** 1035 (1981).
- Ivory, C. F. and R. L. Bratzler, "The Measurement of Rate Coefficients in Slurry Reactors," *Chem. Engng. Commun.*, **10** 293 (1981).
- Ivory, C. F. and R. L. Bratzler, "Error Incurred in Gel Permeation Chromatography by Using the Elution Peak Volume in Lieu of the Elution Mean Volume in the Calculation of K_{av} ," *J. Chromatogr.*, **198** 354 (1981).
- Ivory, C. F., "Continuous Flow Electrophoresis. The Crescent Phenomenon Revisited-I. Isothermal Effects," *J. Chromatogr.*, **195** 165 (1980).

Proceedings

- Dutta, P., K. Horiuchi, H. Cui and C. F. Ivory Proceedings of the IMECE'04, "Band Deformation at a T-Junction While Electrofocusing in a Dog-Leg Microchannel."
- Dutta, P., K. Horiuchi, H. Cui and C. F. Ivory Proceedings of the IMECE'03, "High Resolution Separation of Proteins in a Polymeric Micro-Fluidic Chip."
- C. F. Ivory and W. A. Gobie, "Zone Electrophoresis in Downstream Processing," *Proceedings of the American Society of Mechanical Engineers*, San Francisco, 1989.
- C. F. Ivory, W. Gobie and R. Turk, "Scale-Up of the Free-Flow Electrophoresis Device," in *Electrophoresis '83*, the Proceedings of the Electrophoresis Society Meeting in Tokyo, Japan, May 9-12, 1983.

11. OTHER PUBLICATIONS.

Book Chapters

- Alternative Focusing Techniques, Ivory, C. F., in the *Handbook of Isoelectric Focusing and Proteomics*, S. Ahuja and D. Garfin, eds., CRC Press 2005
- Chapter 9:** "Electrophoresis of Proteins: Batch and Continuous Methods, in *The Encyclopedia of Bioprocess Technology: Fermentation, Biocatalysis and Bioseparations*, M. Flickinger and S. Drew, eds., John Wiley and Sons, Inc. 1999
- Chapter 16: Electrically Driven Separations Processes. Analytical and Preparative Methods, in *Downstream Processing in Biotechnology*, J. Asenjo, ed., Marcel Dekker, Inc., New York 1990.

Journal Editorship (single issue)

- Separation Science and Technology*, Special Issue on Bioseparations, C. F. Ivory, Special Issue Editor, Marcel Dekker, Inc., New York 1988.

Patents and Patent Applications

- Patent Disclosure for "Velocity Gradient Focusing," C. F. Ivory, February 2004. Provisional Patent Application Filed May 2004.
- US Patent Application "Preparative Dynamic Field-Gradient Focusing" C. F. Ivory, N. Tracy, Filed February 2004.
- US Patent Application N°60/446,287 "Dynamic Field Gradient Focusing (Continuation)," C. F. Ivory, Z. Huang and F. Scheutze, Filed Feb. 2003.

- US Patent N° 6,277,258 “Dynamic Field Gradient Focusing,” C. F. Ivory, Z. Huang and F. Scheutze, Filed June 1999, Issued, August. 2001.
- U.S. Patent N° 5,071,536 “High Resolution Continuous Flow Electrophoresis,” C. F. Ivory. Filed November 25, 1985; Issued December 10, 1991.
- U.S. Patent N° 5,200,050 “New Apparatus for Preparative Free-Fluid Electrophoresis,” C. F. Ivory and W. A. Gobie, Filed February 1991, Issued May 30, 1993.
- U. S. Patent N° 5,298,143 “*Electrophoretic Processing*”
Inventors: C. F. Ivory and W. A. Gobie. Issued March 29, 1994.
- Patent Disclosure for “Zone Electric Field Gradient Focusing,” W. A. Gobie and C. F. Ivory, May 1992.
first Office Action, February 16, 1993.
- Patent Disclosure for a “New Apparatus for Electrodialysis,” W. A. Gobie and C. F. Ivory, February 1991.
- Patent Disclosure for a “Method for Ion-Selective Ultrafiltration,” W. A. Gobie and C. F. Ivory, January 1991.
- Patent Disclosure for an apparatus for “Continuous Centrifugal Field-Flow Fractionation,” C. F. Ivory and R. L. Zollars, August 1989.
- Patent Disclosure for an apparatus for “Continuous Counter-Current Electro-Chromatography,” C. F. Ivory and W. A. Gobie, October 1988.
- Patent Disclosure for enhancements to invention developed under NSF Grant No. CPE81-05154.
“Continuous Flow Electrophoresis Device with Recycle Ports, Regenerator Ports and Multiple Feed Ports,” C. F. Ivory, November 1984.

12. GRADUATE THESES DIRECTED.

- Huang, Z., *Automated Control of Dynamic Focusing*, Ph.D., WSU (2001).
- Huang, Z., *Dynamic Focusing in an Electric Field Gradient*, M.S., WSU (1999)
- Pant, Kapil, *Linear Stability Analysis of a Hybrid Centrifuge Rotor*, M.S., WSU (1997).
- Becker, Michael, *Protein Focusing in an Electric Field Gradient*, M.S., WSU (1997).
- Greenlee, Robert, *Zone Focusing in Conductivity Gradients*, M.S., WSU (1995).
- Koegler, Wendy, *Zone Electric Field Focusing*, M.S., WSU (1994).
- Church, Todd, *Zone Focusing via Electro-Ultrafiltration*, M.S., WSU (1994).
- Micinski, Stanley, *Electro-Extraction*, Ph.D., WSU (1994).
- Gilmartin, Michael, *A Hybrid Rotor for Centrifugal Separations*, M.S., WSU (1993).
- Adams, Glenn, *In-Situ Oxidation of Organic Waste*, M.S., WSU (1992).
- Micinski, Stanley, *Electro-Extraction*, M.S., WSU (1989).
- Henneford, Craig W., *Electrochromatography*, M.S., WSU (1989).
- McDonald, Craig A., *Field Flow Fractionation*, M.S., WSU (1989).
- Beckwith, James B., *Hydrodynamic Stability of the Continuous Flow Electrophoresis Device*, Ph.D., Notre Dame (1987).
- Dall, Liese A., *Kinetically Enhanced Transport in Carrier-Mediated Membranes*, Ph.D., Notre Dame (1987).
- Gobie, William A., *Continuous Flow Electrophoresis with Recycle*, Ph.D., Notre Dame (1987).
- Gallagher, Paul M., *The Effect of DC Electric Fields on Carrier-Mediated Transport*, Ph.D., Notre Dame (1986).
- Athayde, Amulya L., *The Effect of AC Electric Fields on Carrier-Mediated Transport*, Ph.D., Notre Dame (1985).
- Turk, Richard S., *Temperature Profiles in the Modified Continuous Flow Electrophoresis Device*, M.S., Notre Dame (1983).

In Progress...

- Bottenus, D., *Nanometer-scale Bioseparations Chips*, MS., WSU (2005).

- Burke, J., *Fractionation of Membrane Proteins using DFGF*, MS., WSU (2005).
- Moreau, S. *Multistage Isotachophoresis*, PhD, WSU (2008).
- Hardin, A.-M., *Protein Transport in Ion Exchange Resins*, PhD., WSU (2007).
- Cui, B., *Staged Isoelectric Focusing on a Microchip*, PhD., WSU (2007).
- Thome, B., *Fractionation of Enantiomer Pairs by Free-Flow Electrophoresis*, PhD., WSU (2005).
- Laird, S., *Couple Dynamic Focusing to Mass Spectrometer for Proteomics*, MS, WSU (2003).
- Tracy, N. *Scale up Dynamic Focusing Column from Micrograms to Milligrams*, PhD, WSU (2006).



Attachment C

TRACY RANKIN
(301) 435-6979
rankint@mail.nih.gov

SUMMARY STATEMENT
(Privileged Communication)

Release Date: 08/19/2003

Application Number: 1 R03 HD045370-01

MCLEAN, DEREK J PHD
WASHINGTON STATE UNIVERSITY
DEPT OF ANIMAL SCIS LAB BLDG
EAST WILSON ROAD PO BOX 646353
PULLMAN WA 991646353

Review Group: CHHD-G (RS)
Population Research Subcommittee

Meeting Date: 08/04/2003
Council: OCT 2003
Requested Start: 01/01/2004

RFA/PA: PAR99-126
PCC: RS -TR

Project Title: Identification of spermatogonial membrane proteins

SRG Action: Priority Score: 287 Percentile: 59.2 #

Human Subjects: 10-No human subjects involved

Animal Subjects: 30-Animals involved - no SRG comments or concerns noted

Project Year	Direct Costs Requested	Estimated Total Cost
1	50,000	70,305
2	50,000	70,305
TOTAL	100,000	140,609

ADMINISTRATIVE BUDGET NOTE: The budget shown is the requested budget and has not been adjusted to reflect any recommendations made by reviewers. If an award is planned, the costs will be calculated by institute grants management staff based on the recommendations outlined below in the COMMITTEE BUDGET RECOMMENDATIONS section.

NEW INVESTIGATOR

RESUME AND SUMMARY OF DISCUSSION: This new NICHD Small Grants Program application is from Washington State University. The PI is Dr. Derek Josef McLean. This is a timely proposal to investigate a significant area of research in male stem cell biology, but unfortunately, one where the proposed technology to be employed does not appear to be sufficiently developed yet to fully answer the questions. As such, there are alternative technologies that are likely to render fruitful outcomes.

There is also significant concern with basing the justification of Aim 2 exclusively on obtaining desired results in Aim 1, especially since the justification for Aim 1 is based solely on one published study in the literature. The single strength of this application is the ability of the PI to identify pseudopod gonocytes from round gonocytes. This needs to be developed further. It may also be prudent if the PI gives some indication where research support funds, if any, are coming from. The R03 grant is insufficient to support animals, salaries, and materials for the work proposed..

DESCRIPTION (provided by applicant): We propose to increase the basic knowledge of spermatogonial stem cell renewal and differentiation by characterization of proteins associated with the cell membrane of spermatogonial stem cells. Spermatogonial stem cells are rare (0.02% of total testis cells in mice) and difficult to study because they have no distinguishing morphological or biochemical characteristics. However, spermatogonial stem cells are essential for the continual production of sperm in the adult male and are the only cells in the postnatal animal that undergo self-renewal throughout life and transmit genes to offspring. The ability to cryopreserve, culture, and transplant these unique cells provides a powerful system to study stem cell biology, preserve individual genomes and modify germ lines. An important step in the isolation and characterization of spermatogonial stem cells is identification of proteins specifically present on these cells. Identification of spermatogonial stem cell specific proteins has been limited due to the inability to isolate a relatively pure population of these cells and the challenge of characterizing proteins from small numbers of cells. In rats, pseudopod gonocytes isolated from day 0-4 animals have spermatogonial stem cell activity in contrast to round gonocytes also found in the testis of day 0-4 animals. This proposal outlines research to compare the integral membrane proteins of pseudopod and round gonocytes with the use of a new electrofocusing technique, dynamic field-gradient focusing (DFGF), that can operate at scales ranging from less than 0.1 μ g to more than 100 μ g of protein and characterize multiple proteins from a sample sequentially. This proposal will test the hypothesis that there is a novel set of proteins on the membrane of pseudopod gonocytes different than the set on the round gonocytes and that these proteins are necessary for the stem cell function of pseudopod gonocytes as determined by successful spermatogonial transplantation. The specific aims of this grant proposal are concentrated on development of techniques to investigate and eventually identify proteins found on the surface of rat spermatogonial stem cells. The identification of novel pseudopod gonocyte proteins will provide an initial characterization of the spermatogonial stem cell membrane proteome and provide new targets to purify spermatogonial stem cells from multiple species.

CRITIQUE NOTE: The sections that follow are, for the most part, unedited, verbatim comments of the reviewers assigned to this application. They are provided to illustrate the range of opinions expressed. The application was discussed and scored by all reviewers present. The attached commentaries may not necessarily reflect the position of the authors at the close of group discussion, nor the final majority opinion of the group. The Resume, however, is the authoritative representation of the outcome of group discussion.

CRITIQUE 1:

SIGNIFICANCE: There is considerable interest and effort in understanding the cell biology of mammalian spermatogenesis. Although there are no model systems that recapitulate complete spermatogenesis *in vitro*, considerable progress towards this goal should be made once the ability to identify, characterize and culture spermatogonial cells in sufficient quantities can be accomplished. One of the major limitations to accomplishing this goal is the availability of specific spermatogonial markers that could be used to identify and purify (by FACS sorting) populations of gonocytes that possess stem cell activity in contrast to gonocytes that lack this activity. Recently, it has been

demonstrated that pseudopod gonocytes from rats have the ability to recolonize a recipient testes giving rise to the initiation of complete spermatogenesis; this is in contrast to round gonocytes which do not have this ability. The problem is separating these two populations in large enough quantities so that molecular analyses can be performed to understand the underlying basis for their stem cell properties.

In this proposal, Dr. Derek Josef McLean proposes to isolate both round gonocytes and pseudopod gonocytes from the rat with the ultimate goal of identifying unique spermatogonial membrane proteins in one cell type versus the other cell type. The identification of those proteins could ultimately be used as markers or for the generation of other reagents (e.g., antibodies) that can be used to isolate larger quantities of these cells for future studies. To accomplish these goals the PI will collaborate with Dr. Cornelius F. Ivory who has developed a new protein electrofocusing technique of dynamic field-gradient focusing (DFGF) that can operate at protein concentrations ranging from less than 100 ng to greater than 100 ug, and can be used to characterize multiple proteins from a sample in a sequential fashion. The identification of novel pseudopod gonocyte proteins will aid in the initial characterization of the spermatogonial stem cell proteome and provide a means to purify these cells.

Clearly, isolation of large numbers of pseudopod gonocytes for subsequent transplantation, biochemical and molecular analyses will represent a substantial step forward in understanding the mechanism of stem cell activity of the male gonad. Such data are not only important to the field of reproductive biology but to several other fields of cellular biology. This subject area, therefore, is of interest and significance. However, some of the experiments proposed in this proposal, while conceptually appropriate, are not backed up with enough information to judge whether they are currently feasible or not, and this dampens enthusiasm for it.

APPROACH: Two specific aims are proposed and both aims are linked conceptually. In the first aim, the PI will isolate, by micromanipulation, the pseudopod and round gonocytes from the rat neonatal testis, and will then transplant them into donors to confirm that the pseudopod gonocytes, in contrast to the round gonocytes, can establish donor-derived spermatogenesis. These experiments will also be performed to ensure that the PI is using the proper identification criteria for isolating the different gonocytes. The PI provides preliminary data showing the two different cell populations in suspension and the criteria that he will use to identify these populations. In these experiments, the PI will select and separate approximately 5,000 cells of the two populations from rats expressing the β -galactosidase transgene (as a marker) using micromanipulation and will transplant them via the efferent ducts into the busulfan-treated testes of recipient rats. Successful colonization and retention of stem cell function will be monitored by β -galactosidase-positive germ cell and sperm production in the recipients. The goal is to reproduce recently published data from another laboratory that dealt with these cell populations, to confirm that the cells after harvesting are functional and to ensure that the PI is using the proper morphological criteria to identify the different populations. In this regard, this aim is somewhat confirmatory, but is clearly necessary for the success of the second aim. The PI should not have a problem performing the transplants, as he has had experience doing this during his post-doc. The ability to identify and separate 5,000 functionally viable cells in a short enough time will be essential for the experiments outlined in the second aim.

In the second aim, the PI proposes to isolate and fractionate pseudopod and round gonocyte membrane proteins using the DFGF procedure. The PI and the co-investigator provide several pieces of preliminary data that speak to the versatility of the DFGF procedure; however, in some of the data they use purified proteins to demonstrate the versatility of this system. More convincing data for the use of this approach in this aim would be the analysis of membrane fractions of a well-characterized cell type (e.g., erythrocytes; neutrophils). In initial experiments, the PI will use purified recombinant Ret protein, a transmembrane protein that displays hydrophobic properties to begin to optimize detergent extraction conditions before the gonocyte membranes are isolated. Again, a more informative experiment might be to spike the Ret protein into a membrane protein mixture from another cell type to get a sense of the complexity of the extraction approaches and DFGF procedures using a mixed

they want a proven separation technique

population of proteins, since this is what the PI will be dealing with when it comes to working with the gonocyte membranes. With respect to the properties of membrane proteins, how does the PI propose to deal with proteins that are not integral membrane proteins but that behave like integral membrane proteins under most extraction conditions? This might confound interpretation of the data as to what proteins are actually membrane proteins. The only way to get at this question might be to go back with specific reagents after the fact (e.g., antibodies) and confirm the localization. This has not been

considered in this proposal and is a very real possibility. This reviewer also has some questions pertaining to the experiments in this aim related to dynamic range balancing. How does the PI propose to alter the conditions of the electrical gradient to demonstrate balancing if there are no markers for those proteins since the goal is to identify markers? In the preliminary data, they use colored protein markers but this cannot be done in the experiments proposed. Finally, perhaps the biggest weakness

of this aim is that the PI provides no estimates of how many cells they will need to get in order to obtain enough material to identify on the gradients, let alone get amino acid sequence. There is no attempt to provide those numbers and this is a real weakness to this aim. They state that they don't anticipate difficulty obtaining adequate amounts of protein for analysis. This is based on what? For example, if they need 20,000 cells to get enough material (considering loss of proteins at different steps and the representation of membrane proteins versus soluble proteins), is this feasible to do by micromanipulation techniques. This aim would be greatly strengthened by providing data that speaks to the feasibility of the experiments.

INNOVATION: An understanding of the molecular mechanisms by which pseudopod gonocytes possess stem cell activity as opposed to round gonocytes should further our knowledge of spermatogenesis, in particular, and stem cell biology, in general. Therefore, the innovation factor is medium to high on this project. The proposed experiments, however, do not challenge existing paradigms but do employ some novel methodologies. As stated above, in its current form, issues of feasibility of several of the aims are called into question, and must be resolved before funding is granted.

INVESTIGATOR: Dr. Derek Josef McLean is a young investigator who has just become an Assistant Professor in the Department of Animal Sciences at Washington State University. He has a good record from his postdoctoral training and clearly has the expertise to perform these experiments. He has obtained a National Research Service Award (NRSA), which is now completed.

ENVIRONMENT: The research environment at Washington State University is excellent and the co-investigators laboratories are well equipped to carry out these experiments. The PI appears to have the appropriate resources to carry out this work, and institutional support appears adequate.

CRITIQUE 2:

SIGNIFICANCE: There is significant need to understand the protein make up of spermatogonial stem cells. This information will undoubtedly shed insight into the mechanisms that govern stem cell differentiation versus holding at steady state and will be useful in the management of patients suffering infertility of unknown causes. Unfortunately, there is also significant skepticism with the possibility that little, if any, information will be gained at the completion of this application because of the immense efforts needed to harvest sufficient material to analyze the putative stem cells at the biochemical level.

APPROACH: This application consists of two aims. In Aim 1, spermatogonial stem cells will be isolated from day four post natal rat testis and selected based on the morphological criterion of whether or not they exhibit pseudopods (true stem cells) versus those that do not (spermatogonia). This rationale is based on one study in the literature that has yet to be confirmed by other investigators in the field. Thus, while possibly interesting, at present the rationale for this aim merits some caution. Moreover, the PI does provide some preliminary studies that it is possible to distinguish cells containing pseudopodia from those that do not in a heterogeneous field, but it would have been more convincing to demonstrate a low power image confirming the level of reliability of such a distinction. This is an important

*same
to
determine
whether*

*we did
this*

consideration because successful completion of both aims depends on this procedure. Finally, it would seem that the isolated cells expressing pseudopodia should be characterized at some level other than phase microscopy to ensure that they represent the spermatogonial component of this mixture. Either ultrastructural studies or some immunostaining protocol should be performed to at least try and characterize the cells. Another concern with this aim is that the PI acknowledges that the pseudopod expressing gonocytes begin to lose their stem cell potential with time, specifically by two hours in culture. Unfortunately, no data are provided as to how many of these cells can be harvested from a four day rat during the critical two hour period. These concerns aside, a strength of this particular aim is that the PI has proven his expertise in performing the transplantation method. Thus, there is optimism that some information will be gained from performing the experiment.

Aim 2 is to purify the membrane fractions from pseudopod and round gonocytes isolated as in Aim 1 for fractionation using DFGF. The only rationale for this aim, however, is that the PI will have first demonstrated in aim one that the pseudopod gonocytes are the true stem cells and as such must contain different protein compositions than the round spermatocytes. But, the question must be asked, what happens if Aim 1 does not yield the desired results? Aside from this major flaw in the design of

Aim 2, there are other significant concerns. First, the DFGF method has not yet been shown to be a viable method to fractionating integral membrane proteins. While the presence of the co-investigator is recognized as a strength, preliminary studies using DFGF to fractionate an isolated membrane sample should have been performed to diminish concern that indeed, it will be possible to fractionate integral membrane proteins using this protocol. Second, and perhaps most alarming, it is not clear how much material will be required to obtain meaningful results. Given the preliminary studies, it is not believable that sufficient numbers of pseudopod and round gonocytes will be isolated routinely to perform a biochemical fractionation.

INNOVATION: If the proposed work is completed, there is high potential that novel proteins, some of which may be uniquely present in the spermatogonial stem cells, will be identified. This in itself will be highly innovative. There is significant concern with the technical aspects of the proposed work, however, that any meaningful results will be obtained. Also, it is possible that the underlying justification of the proposed work is not valid, given that it is based solely on one manuscript in the literature. It is quite possible, for example, that what distinguishes a stem cell from a gonocyte does not reside in the plasma membrane.

INVESTIGATOR: The PI is an accomplished young investigator who is one of the few individuals in the U.S. who has mastered the art of spermatogonial transplantation first pioneered by Dr. Ralph Brinster. The co-investigator is a senior investigator who has helped develop and continues to work on the technology to be employed in the present application, dynamic field-gradient focusing.

ENVIRONMENT: The environment appears outstanding to perform the work. The co-investigator is a developer of the technology to be employed and it would seem that he will be instrumental in trying to make it work for stem cell characterization.

THE FOLLOWING RESUME SECTIONS WERE PREPARED BY THE SCIENTIFIC REVIEW ADMINISTRATOR TO SUMMARIZE THE OUTCOME OF DISCUSSIONS OF THE REVIEW COMMITTEE ON THE FOLLOWING ISSUES:

PROTECTION OF HUMAN SUBJECTS: Human subjects are not part of this application. Code 10.

VERTEBRATE ANIMALS: Vertebrate animals are used and there are no concerns or comments. Code 30.

COMMITTEE BUDGET RECOMMENDATIONS: The requested budget (\$50,000) is recommended. However, it is not clear how funds will be available to purchase animals and materials to perform the

work after salary support is taken care of to pay the PI, co-investigator, and two graduate students. This is a major area of concern.

Ad hoc or special section application percentiled against "Total CSR" base.

NOTICE: The NIH has modified its policy regarding the receipt of amended applications. Detailed information can be found by accessing the following URL address:
<http://grants.nih.gov/grants/policy/amendedapps.htm>

NIH announced implementation of Modular Research Grants in the December 18, 1998 issue of the NIH Guide to Grants and Contracts. The main feature of this concept is that grant applications (R01, R03, R21, R15) will request direct costs in \$25,000 modules, without budget detail for individual categories. Further information can be obtained from the Modular Grants Web site at <http://grants.nih.gov/grants/funding/modular/modular.htm>

MEETING ROSTER

Population Research Subcommittee
National Institute of Child Health and Human Development Initial Review Group
NATIONAL INSTITUTE OF CHILD HEALTH AND HUMAN DEVELOPMENT
CHHD-G (RS) 1
August 04, 2003 - August 05, 2003

CHAIRPERSON

RADOVICK, SALLY, MD
ASSISTANT PROFESSOR
DEPARTMENT OF PEDIATRICS
THE UNIVERSITY OF CHICAGO
WYLER CHILDREN'S HOSPITAL
CHICAGO, IL 60615

MEMBERS

BARNHART, KURT T, MD, MSCE *
ASSISTANT PROFESSOR
DEPARTMENT OF OBSTETRICS AND GYNECOLOGY
UNIVERSITY OF PENNSYLVANIA MEDICAL CENTER
PHILADELPHIA, PA 19104

BISHOP, COLIN E, PHD
PROFESSOR
DEPARTMENT OF OBSTETRICS AND GYNECOLOGY
BAYLOR COLLEGE OF MEDICINE
HOUSTON, TX 77030

CAMERON, JUDY L *
ASSOCIATE SCIENTIST
DEPARTMENT OF PSYCHIATRY
PRIMATE RESEARCH LABORATORY
UNIVERSITY OF PITTSBURGH
PITTSBURGH, PA 15213

DELEON, PATRICIA A., PHD *
PROFESSOR
DEPARTMENT OF BIOLOGICAL SCIENCES
UNIVERSITY OF DELAWARE
NEWARK, DE 19718

DIAS, JAMES A., PHD
RESEARCH SCIENTIST
DEPARTMENT OF HEALTH
DIVISION OF MOLECULAR MEDICINE
WADSWORTH CENTER
ALBANY, NY 12208

FITZGERALD, MARYPAT, MD *
ASSISTANT PROFESSOR
ASSISTANT PROFESSOR
DEPARTMENT OF OBSTETRICS AND GYNECOLOGY
MEDICINE AND RECONSTRUCTIVE SURGERY
LOYOLA UNIVERSITY MEDICAL CENTER
MAYWOOD, IL 60153

GOLDEN, ANDY, PHD *
RESEARCHER
NIDDK, NATIONAL INSTITUTES OF HEALTH
LABORATORY OF BIOCHEMISTRY AND GENETICS
BETHESDA, MD

GUZICK, DAVID S, MD *
PROFESSOR AND CHAIR
SCHOOL OF MEDICINE
UNIVERSITY OF ROCHESTER
ROCHESTER, NY 14642

KOPF, GREGORY S., PHD *
PROFESSOR
DISCOVERY CONTRACEPTION
WOMEN'S HEALTH RESEARCH INSTITUTE
WYETH AYERST RESEARCH
PHILADELPHIA, PA 19426

MOENTER, SUZANNE M., PHD *
ASSOCIATE PROFESSOR
DEPARTMENTS OF MEDICINE AND CELL BIOLOGY
DIVISION OF ENDOCRINOLOGY AND METABOLISM
UNIVERSITY OF VIRGINIA SCHOOL OF MEDICINE
CHARLOTTESVILLE, VA 22908

MYERS, EVAN R, MD, MPH *
ASSOCIATE PROFESSOR
DEPARTMENT OF OBSTETRICS AND GYNECOLOGY
SCHOOL OF MEDICINE
DUKE UNIVERSITY
DURHAM, NC 27710

SCHLEGEL, PETER N, MD
ASSOCIATE PROFESSOR
DEPARTMENT OF UROLOGY
WEILL MEDICAL COLLEGE OF CORNELL UNIVERSITY
NEW YORK PRESBYTERIAN HOSPITAL
NEW YORK, NY 10021

SEIFER, DAVID B, MD *
PROFESSOR
UMDNJ RWJ MEDICAL SCHOOL
OB, GYN AND REPRODUCTIVE SCIS
NEW BRUNSWICK, NJ 08901

SUAREZ-QUIAN, CARLOS A., PHD
ASSOCIATE PROFESSOR
DEPARTMENT OF CELL BIOLOGY
GEORGETOWN UNIVERSITY
WASHINGTON, DC 20007

WELLS, DAGAN, PHD *
SENIOR SCIENTIST
THE INSTITUTE FOR REPRODUCTIVE MEDICINE &
SCIENCE
SAINT BARNABAS MEDICAL CENTER
WEST ORANGE, NJ 07052

WOLGEMUTH, DEBRA J., PHD *
PROFESSOR
DEPARTMENT OF GENETICS AND DEVELOPMENT
COLUMBIA UNIVERSITY COLLEGE OF PHYSICIANS AND
SURGEONS
NEW YORK, NY 10032

WOODRUFF, TERESA K., PHD
ASSOCIATE PROFESSOR
DEPARTMENT OF NEUROBIOLOGY AND PHYSIOLOGY
NORTHWESTERN UNIVERSITY
EVANSTON, IL 60208

SCIENTIFIC REVIEW ADMINISTRATOR

RANHAND, JON M., PHD
SCIENTIST REVIEW ADMINISTRATOR
DIVISION OF SCIENTIFIC REVIEW
NATIONAL INSTITUTE OF CHILD HEALTH
AND HUMAN DEVELOPMENT
BETHESDA, MD 20892

GRANTS TECHNICAL ASSISTANT

NEELY, VERONICA D.
GRANTS TECHNICAL ASSISTANT
DIVISION OF SCIENTIFIC REVIEW
NATIONAL INSTITUTE OF CHILD HEALTH
AND HUMAN DEVELOPMENT, NIH
BETHESDA, MD 20892

* Temporary Member. For grant applications, temporary members may participate in the entire meeting or may review only selected applications as needed.

Consultants are required to absent themselves from the room during the review of any application if their presence would constitute or appear to constitute a conflict of interest.